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X-ray Crystal Structure and EPR Spectra of "Arsenite-Inhibited" *Desulfovibrio gigas* Aldehyde Dehydrogenase: A Member of the Xanthine Oxidase Family

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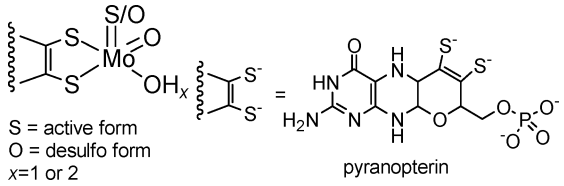
Aldehyde dehydrogenase (ADH, or aldehyde oxidoreductase, also known as MOP) from *Desulfovibrio gigas* belongs to the xanthine oxidase (XO) family of mononuclear molybdenum enzymes.[1] The crystal structure contains a Mo coordinated by one pyranopterin, two oxo ligands, and one hydroxo/water molecule,[1d] consistent with X-ray structures of other XO family members (Chart 1). This inactive form does not contain a cyano-lysable sulfido ligand believed to be present in the active enzyme used for crystallization. However, this type of coordination is essentially the same upon resulfuration in both oxidized and reduced states.[1e]

The inhibition of XO by arsenite was reported over 70 years ago and has been studied extensively.[2] The arsenite ion reacts at the molybdenum site, and the enzymatic activity is lost. The Mo(V) ion EPR signal obtained upon reduction of the as isolated XO incubated with arsenite was shown to have both hyperfine and quadrupole couplings of a single arsenic nucleus ($I = 3/2$) to Mo(V) ($S = 1/2$).[2a] These authors propose a structure in which the arsenic and the molybdenum atoms are connected by a sulfido bridge. A similar conclusion was obtained from EXAFS studies of reduced XO samples,[2b] while other authors proposed a structure with a double bridge (oxo and sulfido).[2c] This work presents the first molecular structure of an arsenite-inhibited complex of a member of the XO family and the EPR properties of a paramagnetic species detected in the reduced form of the active enzyme in the presence of arsenite.

ADH was purified from *D. gigas* as described by Moura et al.[3] Crystals were obtained under similar conditions as reported previously.[1b,4] After the crystals reached their maximum size, the crystallization buffer was replaced with a soaking buffer depleted of 2-propanol and containing $NaAsO_2$.[4] A single dataset was collected at 100 K using synchrotron radiation.[4] The reported 1.28 Å resolution structure, in the inactive desulfo form,[1c] stripped of water and 2-propanol molecules, was used as a starting model for refinement. The refined structure is very similar to the 1.28 Å resolution structure with the exception of small details consisting mainly of different occupancies of chemical groups. Most notably, the 2-propanol molecules present in the original structure dissociate from the protein molecules apparently without destroying the crystalline lattice.

However, the replacement of the crystallization solution with a buffer depleted of 2-propanol decreases the quality of the crystals as demonstrated by the increase in the mosaicity when compared to the original crystals. Furthermore, dissociation of about half of the equivalents of molybdenum occurred. Difference Fourier electron density maps and temperature factor analysis suggest an occupancy of 60%, which was subsequently used in refinement. Loss of Mo has not been observed in the crystal structures of ADH from *D. gigas* reported before. It has been reported, however, for

Chart 1. Schematic Structure of the Active Site in XO Family Enzymes.



the homologous ADH from *Desulfovibrio desulfuricans*[5a] (the structure was refined with an occupancy of 0.5 for Mo)[5b] and is common for other enzymes of the xanthine oxidase family.[1a] It is likely that the 2-propanol present in *D. gigas* ADH stabilizes its active site, preventing the loss of molybdenum. These findings give further evidence that the gorge leading away from the molybdenum is an entry/exit path of substrates and products, as has been proposed.[1d] Attempts to crystallize the complex from the native protein, in the presence of 2-propanol and arsenite, failed to show the formation of an arsenite-inhibited complex, probably due to the fact that in these experiments 2-propanol blocks the entrance pathway of the arsenite molecule.

In the initial electron density maps of the structure described here,[6] density is found at 3.3 Å from Mo at 3.5σ in a weighted $2F_o - F_c$ omit map. The density was modeled as an arsenic atom with three bound oxygens. It has been shown that AsO_2^- associates with water, yielding a mixture of species, i.e., $[AsO(OH)_2]^-$, $[AsO_2(OH)]^{2-}$, and $[AsO_3]^{3-}$.[7] The arsenite moiety is bound to the molybdenum atom through one of its oxygen atoms. One of the other oxygen atoms is found in the proximity of glutamate 869, a conserved residue that is essential for catalysis. Figure 1 shows the geometry of the molybdenum active site and the electron density in the vicinity of the Mo center, as well as the geometry of the structure reported previously.[1c]

Figure 2 shows two EPR spectra of arsenite-incubated ADH samples,[4] reduced with an excess of dithionite, obtained at different temperatures.[8] Simulation of the Mo(V) signal at 140 K (Figure 2) was obtained assuming an Mo(V) species ($S = 1/2$) coupled to an arsenic atom ($I = 3/2$) by both hyperfine and quadrupolar interactions. The EPR parameters show slight differences with respect to the ones obtained for XO,[2a] which can be ascribed to slight structural changes in the active sites. These differences are not significant, indicating that the structure of the Mo(V)—arsenite complexes obtained in both enzymes are similar. Samples exchanged with buffered D_2O solution showed no differences with the normal samples (not shown), which suggests that the first coordination sphere of the Mo(V) ion is not occupied by water molecules.

The 20 K spectrum shows, in addition to the Mo(V) signal, the EPR signal associated with the two [2Fe—2S] clusters (Fe/S I and Fe/S II). The EPR signal associated with the Mo(V) species

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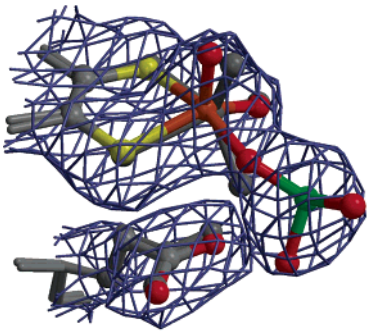

Figure 1. Geometry of the arsenite-inhibited molybdenum catalytic center. The 1.28 Å resolution ADH structure[1c] is shown in gray, and the arsenite-inhibited structure is color-coded (Mo in orange, As in green, C in gray, S in yellow, and O in red). The Mo−O−As angle is around 125°.

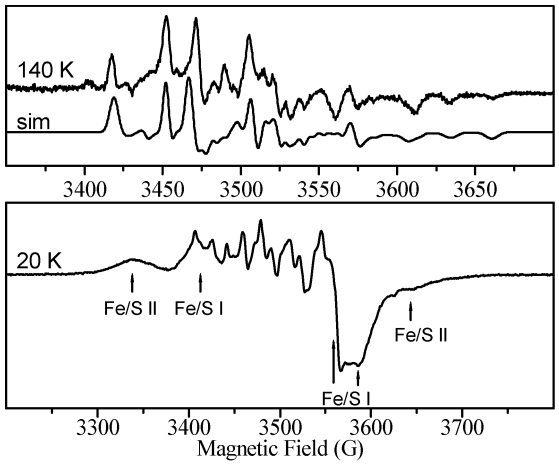

Figure 2. Arsenite-inhibited ADH at 140 and 20 K with a simulation of the high-temperature spectrum (g_1 = 1.922(12), g_2 = 1.979(6), g_3 = 1.972(9), A_1 = 120, A_2 = 40, A_3 = 136, P_1 = −9, P_2 = 19, P_3 = −10). g-value uncertainties = ±0.001. Half-height line widths are in parentheses. A and P are in MHz. g and A tensors are assumed to be collinear, and the P tensor is rotated an Euler angle $\alpha = 30°$. The simulation was performed with a Hamiltonian $H = \beta H \cdot g \cdot S + S \cdot A \cdot I + I \cdot P \cdot I$, where all symbols have their usual meaning.[4]

shows temperature-dependent splittings produced by magnetic coupling with Fe/S I, as observed in dithionite-reduced samples of *D. gigas* ADH.[1a] The Mo(V) signal overlaps with the signal of Fe/S I, making a clear evaluation of the EPR parameters difficult. However, the positions of the signals indicate that both Fe/S I and II signals are identical to those obtained in reduced samples of *D. gigas* ADH.[1a,3] After air reoxidation of the dithionite-reduced samples, the Fe/S cluster signals are not detectable, whereas the Mo(V) signal remains detectable, which indicates that the separation between the redox couples (Mo(VI)−Mo(V) and Mo(V)−Mo(IV)) is larger than that observed in normal *D. gigas* ADH. Figure 3 shows the saturation behavior of the Mo(V) signal at 140 K in reduced and air-reoxidized samples. The Mo(V) species in the presence of reduced Fe/S I are saturated at higher powers with respect to the oxidized samples, which indicates that Fe/S I not only splits the Mo(V) resonance lines but also enhances its relaxation properties.

In conclusion, we have shown that (1) arsenite coordinates to the molybdenum via an oxygen bridge in the desulfo form of the enzyme; (2) the proposed chemical pathway for electron transfer is conserved; and (3) although the presented structure represents the inactive form of the enzyme, the site of binding of the arsenite is at the position of substrate binding, indicating that the inhibition mechanism occurs through a competition with the substrate.[1e,9]

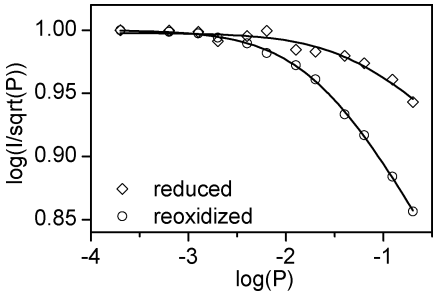

Figure 3. Normalized power dependence plot of reduced and reoxidized arsenite-inhibited EPR signal. The equation $y = P_1 - (P_2/2) \cdot \log(1 + P/P_3)$ was fitted to the data with P_1 = 0.998(2), P_2 = 0.15(5), and P_3 = 0.05(3) for the reduced signal and P_1 = 1.0002(7), P_2 = 0.279(9), and P_3 = 0.021(2) for the reoxidized signal. The intensity of the 3450 G peak (depicted in Figure 2) was used for the data, but all the strong peaks show the same saturation behavior. Microwave power (P) is given in W.

Furthermore, the coordination mode of the arsenite suggests that the substrate reacts with the labile water ligand of the Mo site to form a Mo−O−C bond instead of a Mo−C bond.[10] We intend to pursue further experiments to establish the relationship between the EPR properties and the geometry in the crystal structure.

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Supporting Information Available: Details on crystallization, X-ray data collection, EPR sample preparation, and measurement and EPR signal simulation (PDF, PDB, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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